

Embryogenesis and Larval Development of the Asteroid *Patiriella regularis* Viewed by Light and Scanning Electron Microscopy

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Abstract. The sea star *Patiriella regularis* (Verrill, 1867) has indirect development through bipinnaria and brachiolaria larvae. Development of this species is typical of asteroids with planktotrophic larvae and takes 9–10 weeks. The embryos develop through a wrinkled blastula and hatch as early gastrulae. In contrast to most asteroids, a third enterocoel forms on the left side of the stomach of the bipinnaria. This structure gives rise to the left posterior coelom; its significance is discussed. We suggest that this coelom is homologous to the trunk coelom in enteropneust embryology. The surface features of the larvae were examined by scanning electron microscopy. Newly hatched gastrulae are covered by cilia, and the bipinnaria have bands of cilia that follow the contours of the larval processes. A previously undescribed plug-like structure positioned on the post-oral surface appears to function as a seal for the mouth. Brachiolaria larvae have three brachiolar arms and a centrally located adhesive disc. Each arm is covered by adhesive papillae. Raised epithelial cells that dot the surface of the papillae and adhesive disc may be batteries of secretory cells. The brachiolar arms have an extracellular coat that may serve as a protective cover for the adhesive surfaces. Competent brachiolaria swim along the substratum and exhibit searching behavior with flexure of the median brachium. They settle on the undersides of natural shell substrata and do not respond to a primary algal film. Shade appears to be an important factor in settlement and metamorphosis in *P. regularis*. Metamorphosis takes 5–6 days, and the post-larvae take up a free existence at a diameter of 450–500 μm . The

indirect development of *P. regularis* contrasts with the lecithotrophic and viviparous modes of development of other *Patiriella* species and provides the comparative basis to determine the ontogenic changes involved with evolution of direct development in the genus. The use of the divergent life histories of *Patiriella* as a model system for the study of evolutionary change in development is discussed.

Introduction

The spinulosan sea star *Patiriella regularis* (Verrill, 1867) is common in New Zealand waters, ranging from the intertidal zone to 100 m depth (Mortensen, 1921; Crump, 1971). This species is a member of the *Patiriella* group of which there are eleven species in the Australia-New Zealand region (Dartnall, 1971; Keough and Dartnall, 1978). A remarkable feature of these asteroids is the diversity of life histories that they exhibit, ranging along a continuum from indirect to direct development (Dartnall, 1971; Lawson-Kerr and Anderson, 1978; Byrne, 1991; Table I). *P. regularis* spawns small eggs and develops indirectly through planktotrophic bipinnaria and brachiolaria larvae (Mortensen, 1921; Crump, 1971). These feeding larvae are typical of the Asteroidea and are considered to be of great antiquity (Strathmann, 1978a). In contrast, all the Australian species examined thus far are direct developers. *P. calcar*, *P. pseudoexigua*, and *P. gunnii* have large yolky eggs and develop directly through a non-feeding planktonic brachiolaria (Lawson-Kerr and Anderson, 1978; Grice and Lethbridge, 1989; Byrne, 1991; Chen and Chen, 1991). *P. exigua* oviposits large eggs that develop through a modified benthic brachiolaria (Lawson-Kerr and Anderson, 1978; Byrne, 1991). At the end of the indirect-direct continuum of development exhibited

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Table I

Life history traits of *Patriella* species from Australia and New Zealand*

Species	Oocyte diameter (mm)	Developmental pattern	Larvae
<i>P. regularis</i>	150	Indirect/planktotrophic	Bipinnaria and brachiolaria
<i>P. gunnii</i>	360	Direct/lecithotrophic	Planktonic brachiolaria
<i>P. calcar</i>	400	Direct/lecithotrophic	Planktonic brachiolaria
<i>P. pseudoexigua</i>	—	Direct/lecithotrophic	Planktonic brachiolaria
<i>P. exigua</i>	400	Direct/lecithotrophic	Benthic brachiolaria
<i>P. vivipara</i>	120	Direct/viviparous	Intraovarian brooder No larva
<i>P. parvivipara</i>	100	Direct/viviparous	Intraovarian brooder No larva

* Data from: Dartnall (1969); Crump (1971); Keough and Dartnall (1978); Lawson-Kerr and Anderson (1978); Byrne (1991); Chen and Chen (1991).

by *Patriella*, are the intraovarian brooders, *P. vivipara* and *P. parvivipara*, which give birth to crawl-away juveniles (Dartnall, 1969; Chia, 1976; Keough and Dartnall, 1978; Byrne, 1991).

Several nomenclatural systems have been suggested for the diverse developmental patterns in the Asteroidea (Chia, 1968, 1974; Oguro *et al.*, 1976, 1988). In one system, development through a bipinnaria and brachiolaria larvae is termed indirect, whereas development only through a brachiolaria is termed direct (for review, Oguro *et al.*, 1988). This system is most appropriate for *Patriella*. Other systems make the distinction between indirect-planktotrophic larvae with a functional gut and direct-lecithotrophic larvae without a functional gut (Chia, 1968). The recent finding, however, of an intermediate pattern of asteroid development, through a larva that has both planktotrophic and lecithotrophic features, obscures this distinction (Bosch, 1989).

Comparative embryology of closely related species is a powerful tool for the investigation of developmental processes in evolution because homologous characters can be compared (Raff, 1987). This approach has attracted renewed interest, particularly with respect to echinoids, where recent studies have revealed that direct development arose through heterochronies in the appearance of adult features (Raff, 1987; Wray and Raff, 1989). Heterochronies, changes in the relative timing or rate of ontogenic events, are considered to be an important means of ef-

fecting evolutionary change (Anderson, 1987). The range of life histories in *Patriella* listed in Table I presents an ideal system with which to investigate the modifications involved with the shift to direct development within a monogeneric group. In *P. vivipara*, direct development is achieved by heterochrony in suppression of larval characters and accelerated development of adult features (Byrne, 1991).

In the evolutionary sequence of developmental change in *Patriella*, the planktotrophic development of *P. regularis* represents the ancestral mode of development in the genus, and, in this investigation, is described in detail. Particular attention is paid to the pattern of larval ciliation and the structure of the larval arms, features often modified in lecithotrophic larvae (Strathmann, 1978a). Settlement behavior and metamorphosis are also described. The ontogeny of *P. regularis* will provide the chronological

Table II

Chronology of development of *Patriella regularis* at 18–22°C

Time	Stage
0	Fertilization
15–60 s	Elevation of fertilization membrane
40–60 min	First cleavage
1–1.5 h	Second cleavage
2–2.5 h	Third cleavage
3 h	Fourth cleavage
3.5–5 h	Early blastula
6–9 h	Wrinkled blastula
15–17.5 h	Late blastula/early gastrula
25 h	Hatching, gastrula with elongating archenteron
30–35 h	Advanced gastrula, budding of mesenchyme cells from terminal expansion of archenteron
45–55 h	Early bipinnaria, enterocoel and stomodeum formation, archenteron bent towards oral surface to complete gut, the posterior enterocoel starts as a thickening of the left side of the archenteron
55–60 h	Bipinnaria, ciliary bands distinct, gut regions differentiate
65–75 h	Bipinnarial arms well-developed, posterior enterocoel is vesicular, posterior elongation of right and left enterocoels, hydropore open, fusion of left enterocoel with posterior enterocoel
4–5 days	Anterior growth of enterocoels into oral hood and fusion to form the axohydrocoel
6 days	Extension of axohydrocoel into oral hood, right and left enterocoels continue to grow posteriorly, posterior enterocoel forms part of the left posterior coelom, formation of the ventral horn
10–14 days	Advanced bipinnaria, fusion of the ventral horn with the right enterocoel, the axohydrocoel with two lateral extensions for brachia
4–6 weeks	Early brachiolaria, growing brachiolar arms
8 weeks	Advanced brachiolaria, arms and adhesive disc well-developed, five lobes of the hydrocoel present, formation of adult primordium and skeleton
9–10 weeks	Larvae competent to metamorphose

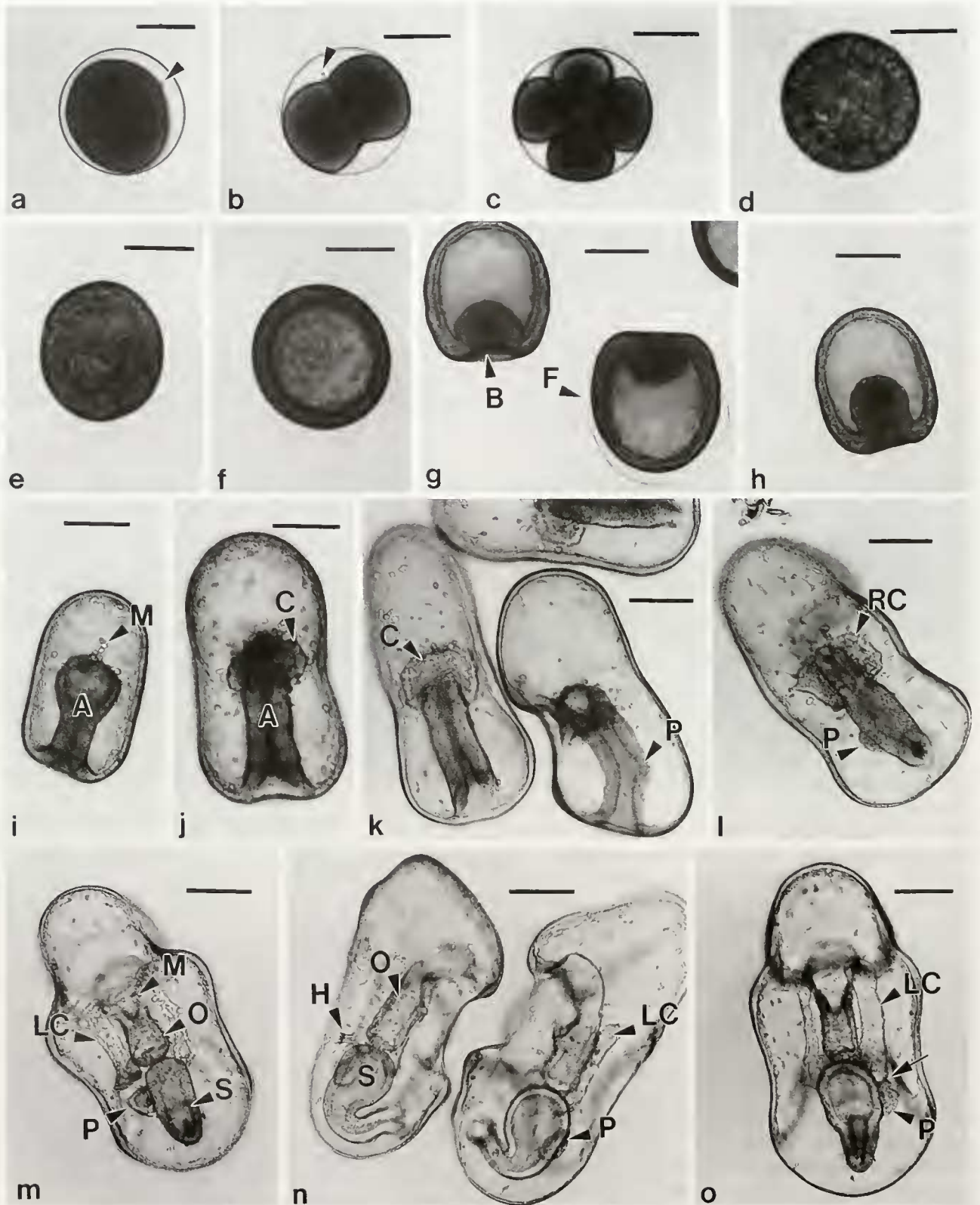


Figure 1. Development through the bipinnaria stage. a. Egg shortly after fertilization with an elevated fertilization membrane and one polar body (arrowhead). b. One hour, first cleavage, two polar bodies are evident (arrowhead). c. One and a half hours, second cleavage. d. Four hours, early blastula. e. Eight hours, wrinkled blastula. f. Sixteen hours, late coeloblastula rotating in membrane. g. Twenty-five hours,

basis required to determine the morphological and heterochronic changes underlying the evolution of direct development within the genus.

Materials and Methods

Specimens of *Patiriella regularis* were collected during slack water at 5–10 m depth from Otago Harbour, New Zealand (45°49.7'S; 170°38.4'E), near the Portobello Marine Laboratory, in January 1990. This species is gonochoric, and ovaries and testes were dissected from mature specimens. The testes were stored dry at 4°C, and the ovaries were placed in a 10⁻⁵ M solution of 1-methyladenine in filtered seawater (Kanatani, 1969). Following ovulation, the ova were transferred to beakers of 1.0 µm filtered seawater and washed gently through several changes of filtered seawater. For fertilization, the eggs were placed approximately one cell deep in 250-ml beakers, and a few drops of diluted sperm were added. After 5–10 min, the eggs were washed in fresh seawater to remove excess sperm, and the fertilized eggs were placed in 5-l beakers of filtered seawater. The cultures were stirred gently by motor-driven paddles. When the embryos attained the swimming gastrula stage, the cultures were filtered and placed in fresh seawater. Thereafter, the seawater in the cultures was changed once a week. Just prior to the early bipinnaria stage, feeding of the larvae commenced. The larvae were fed from unialgal cultures of the flagellate *Dunaliella primolecta* Butcher. The temperature of the culture room ranged from 18° to 22°C.

Embryogenesis and larval development of *Patiriella regularis* were documented by light and scanning electron microscopy (SEM). For SEM, the embryos and larvae were fixed in 2.5% glutaraldehyde in 0.45 µm filtered seawater for 1 h at room temperature. Once the bipinnaria stage was attained, larvae fixed by this method were first relaxed in 6.8% MgCl₂ in distilled water before being placed in the primary fixative. Following primary fixation, the specimens were washed in 2.5% sodium bicarbonate (pH 7.2) and post-fixed in 2% OsO₄ in 1.25% sodium bicarbonate for 1 h at room temperature. The specimens were then washed in distilled water and dehydrated in

ethanol. After dehydration, the specimens were critical point dried, sputter coated, and viewed with a Joel JSM-35C scanning electron microscope. In addition, the fixation method of Barker (1978a) was also used. According to this method, larvae placed in a small drop of seawater were initially fixed by the addition of Bouin's fluid. The larvae were then transferred to 3% glutaraldehyde in 0.2 M cacodylate buffer for 1 h at room temperature. Following a rinse in the same buffer, the specimens were post-fixed in 2% OsO₄ in cacodylate buffer. Although the introduction of Bouin's caused the larvae to contract slightly, this method resulted in good preservation of the extracellular coat of the larvae. After fixation, the larvae were rinsed in distilled water and processed as described above.

Results

Spawning

Spawning of *Patiriella regularis* was observed *in situ* on 25 January 1990. Approximately 20 individuals, both males and females, were observed releasing gametes. The sperm exited from the gonopores as a narrow plume that dissipated 5–10 cm above the spawning individual. For the females, the eggs rolled on to the aboral surface after exiting from the gonopore. The shortest distance between spawning individuals ranged from 0.5 to 1.0 m, while the longest distance ranged from 4 to 5 m. These observations were recorded during the day under sunlit conditions and coincided with slack water.

In the laboratory, the ovaries of *Patiriella regularis* exhibited a long hormone-dependent period. It took 3–5 h before oocyte maturation; ovulation and spawning was induced by 1-methyladenine. The spawned ova were green and 150 µm in diameter (±9 µm; n = 20).

Embryogenesis

The chronology of the development of *Patiriella regularis* is outlined in Table II. A fertilization membrane forms 15–60 s after the introduction of sperm into the beakers containing ova, and the two polar bodies are given off within 20 min (Fig. 1a, b). Cleavage is radial and ho-

hatching gastrulae. B, blastopore; F, fertilization membrane. h. Twenty-five hours, swimming gastrula. i. Thirty-five hours, advanced gastrula, mesenchyme cells (M) are budding off into the blastocoel. A, archenteron. j. Forty-five hours, early bipinnaria, right and left enterocoels are starting to form (C). A, archenteron. k. Forty-seven hours, bipinnaria, enterocoels are forming (C), archenteron is complete. A small bulge on the archenteron is the beginning of the posterior enterocoel (P). l. Fifty-five hours, the right (RC) and left enterocoels grow posteriorly. On the left side of the archenteron, a small group of cells form the posterior enterocoel (P). m. Seventy hours, dorsal view. LC, left enterocoels; M, mouth; P, posterior enterocoel; O, oesophagus; S, stomach. n. Seventy hours, bipinnaria in side view, gut regions and hydropore (H) are evident. LC, left anterior enterocoel; O, oesophagus; P, posterior enterocoel; S, stomach. o. Seventy-five hours, ventral view. The arrow points to the fusing left anterior (LC) and posterior (P) enterocoels. The right and left anterior enterocoels have started to grow into the oral hood. Scale bars = 100 µm.

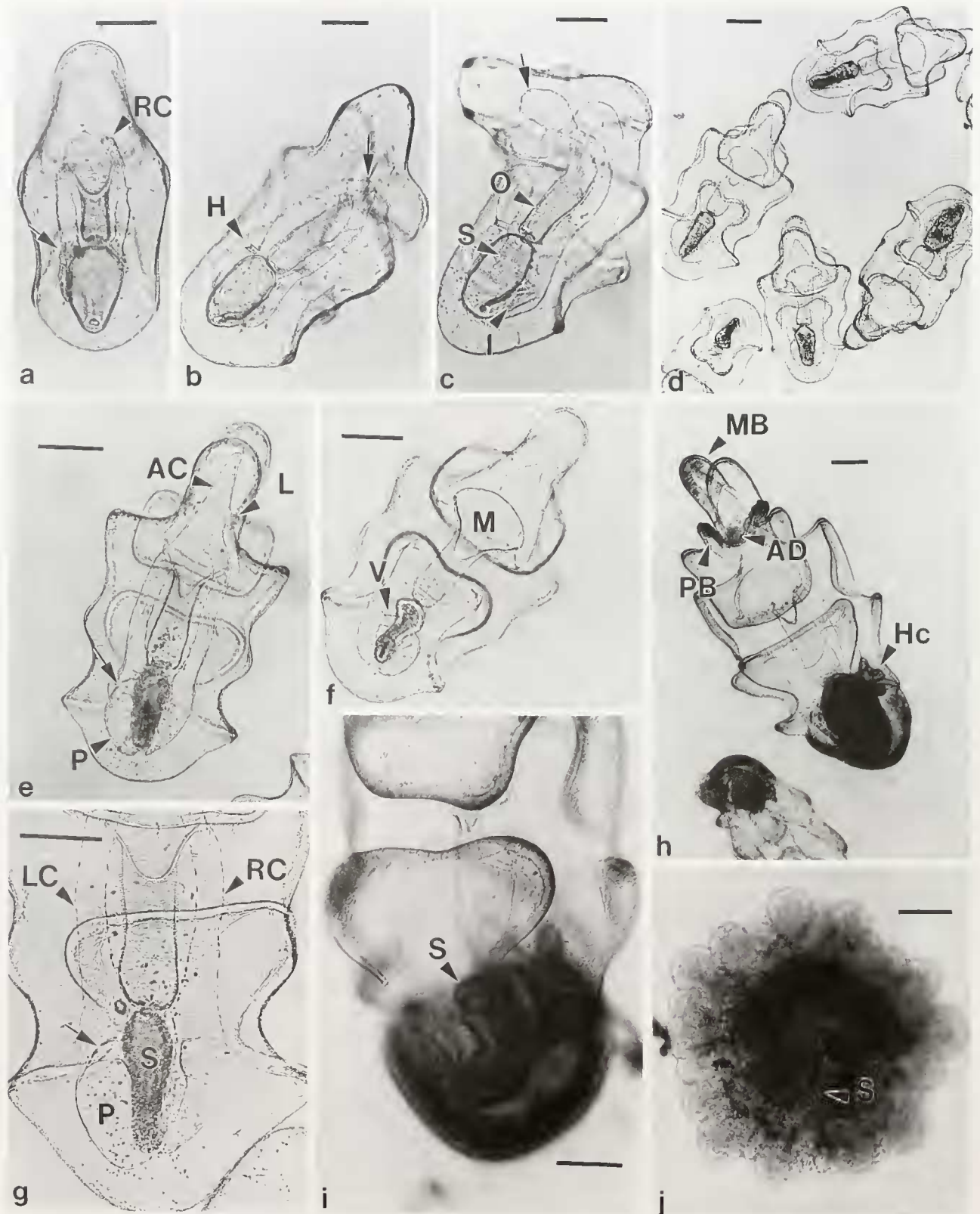


Figure 2. Development through metamorphosis. a. Eighty-eight hours, dorsal view. Growth of the right enterocoel (RC) into the oral hood. At the position where the two left enterocoels have met, tissue derived from fusion of their end walls is evident (arrow). b. Four and a half days, side view of a bipinnaria showing

loblastic (Fig. 1b–d). The first cell division occurs 40–60 min after fertilization, and the early blastula stage is reached within 4 h. By 4 h, asynchrony was evident in all cultures, with some embryos at a more advanced stage than others. Five hours post-fertilization, the embryos are well-developed blastulae 164 μm in diameter ($\pm 7 \mu\text{m}$; $n = 10$) (Fig. 1d). The blastulae rotate within their close-fitting fertilization membranes, propelled by their ciliary covering. Blastular wrinkling starts 6 h after fertilization with folding of the blastoderm into the blastocoel. The furrows of the wrinkled blastulae are most apparent in 8-h larvae (Fig. 1e). Subsequently, the furrows smooth out and 18-h cultures contain late blastulae with a smooth surface and early gastrulae (Fig. 1f, g). Hatching ensues through rupture of the fertilization membrane, and the gastrulae become free-swimming larvae (Fig. 1g, h). At hatching, the larvae are round to elongate and have a shallow blastopore. They continue to elongate with growth of the archenteron into the blastocoel. The blind end of the archenteron expands and mesenchyme cells detach from its tip, moving into the blastocoel (Fig. 1i). At this stage, the larvae are 197 μm long ($\pm 1.2 \mu\text{m}$, $n = 10$).

Early bipinnaria are present by the end of the second day. The right and left enterocoels form as pouches off the expanded tip of the archenteron (Fig. 1j). A shallow stomodeum is present, and the blind end of the archenteron bends towards the oral surface. During this stage, the posterior region bends ventrally, and from this time the blastopore can be regarded as the larval anus. By 55 h, the archenteron fuses with the stomodeal invagination, thereby completing the larval gut (Fig. 1k). With development of the ciliated bands, algal food was introduced into the cultures. The larvae now have a distinct peroral hood region. In addition, a shallow evagination, destined to form the posterior enterocoel, is evident on the left-hand wall of the archenteron; this soon grows to form a small thickening of cells (Fig. 1k, l).

By the end of the third day, the bipinnaria are feeding and have well-defined pre- and postoral ciliary bands. At this stage, the bipinnarial processes—lateral and anterior

projections of the larval body wall—start to form. The regions of the gut differentiate with the expansion of the stomach and the separation of the stomach from the oesophagus by the cardiac sphincter (Fig. 1m). In three-day-old bipinnaria, the right and left enterocoels increase in length as they grow posteriorly, and the hydropore exits on the dorsal surface (Fig. 1n). During the fourth day of development, the small thickening on the archenteron wall grows to form a solid ball of cells attached to the stomach. A central cavity forms in this structure, thereby forming a posterior enterocoel on the left side of the larvae (Fig. 1n, o). This posterior enterocoel increases in size and is a conspicuous feature of all the larvae examined from five different cultures. When the advancing left anterior enterocoel reaches the posterior enterocoel, the two enterocoels fuse (Figs. 1o, 2a). In some larvae, fusion of the two left coelomic pouches was complete 75 h after fertilization. With subsequent development, it was evident that the posterior enterocoel forms part, if not all, of the left posterior coelom. Where the two enterocoels meet, a partition derived from fusion of their tissues forms (Fig. 2e, g). During the fourth day of development, the right and left enterocoels extend anteriorly into the oral hood (Figs. 1o, 2a). The larval length is now 630 μm ($\pm 5.8 \mu\text{m}$; $n = 10$).

In 4.5 day larvae, the anterior extensions of the right and left enterocoels fuse to form the axohydrocoel in the oral hood (Fig. 2b). This anterior coelom grows to form an extension into the hood where the median-dorsal process develops (Fig. 2c–e). Five-day-old larvae are well-developed bipinnaria and the ciliary tracts increase in length following the edges of the bipinnarial processes. The bipinnaria exhibit muscular movements including contraction of the cardiac sphincter and dorsal and ventral flexure of the oral hood, which results in broadening and closure of the oral cavity (Fig. 2c). Internally, the fused left enterocoels extend below the gut, while growth of the right enterocoel is slower. The partition derived from fusion of the two left enterocoels divides the left enterocoel into anterior and posterior regions (Fig. 2e, g). Partition

the anterior coelom (arrow) in the oral hood formed through fusion of the right and left enterocoels. H, hydropore. c. Six days, bipinnaria from the side, exhibiting dorsal flexure, the anterior coelom has grown into the oral hood (arrow). I, intestine, O, oesophagus, S, stomach. d. Four-week-old culture containing late bipinnaria. e. Ten days, dorsal view, late bipinnaria/early brachiolaria. The anterior coelom (AC) has grown to form the lumen of the future median brachium, two small lateral branches at the base of this coelom (L) are destined to be the coelomic lumina of the posterior brachia. The left posterior enterocoel (P) has grown below the gut. A septum-like structure (arrow), partitions the left coelom into anterior and posterior sections. f. Four weeks, ventral view, early brachiolaria, the ventral horn (V) of the left posterior coelom has grown around the gut and fused with the right enterocoel. M, mouth. g. Four weeks, detail of the septum (arrow) dividing the left anterior (LC) and posterior coelom (P). RC, right enterocoel, S, stomach. h. Eight weeks, ventral view, late brachiolaria, the median (MB) and posterior (PB) brachia and the adhesive disc (AD) are well-developed. The lobes of the hydrocoel (Hc) are evident. i. Eight weeks, ventral view, adult primordium of a late brachiolaria, primary spicules (S) lie along the lobes of the hydrocoel. j. Nine weeks, metamorphosing larva from the aboral surface. S, skeleton. Scale bars: a,b,c,g,i,j = 100 μm . Scale bars: d,e,f,h; = 150 μm .

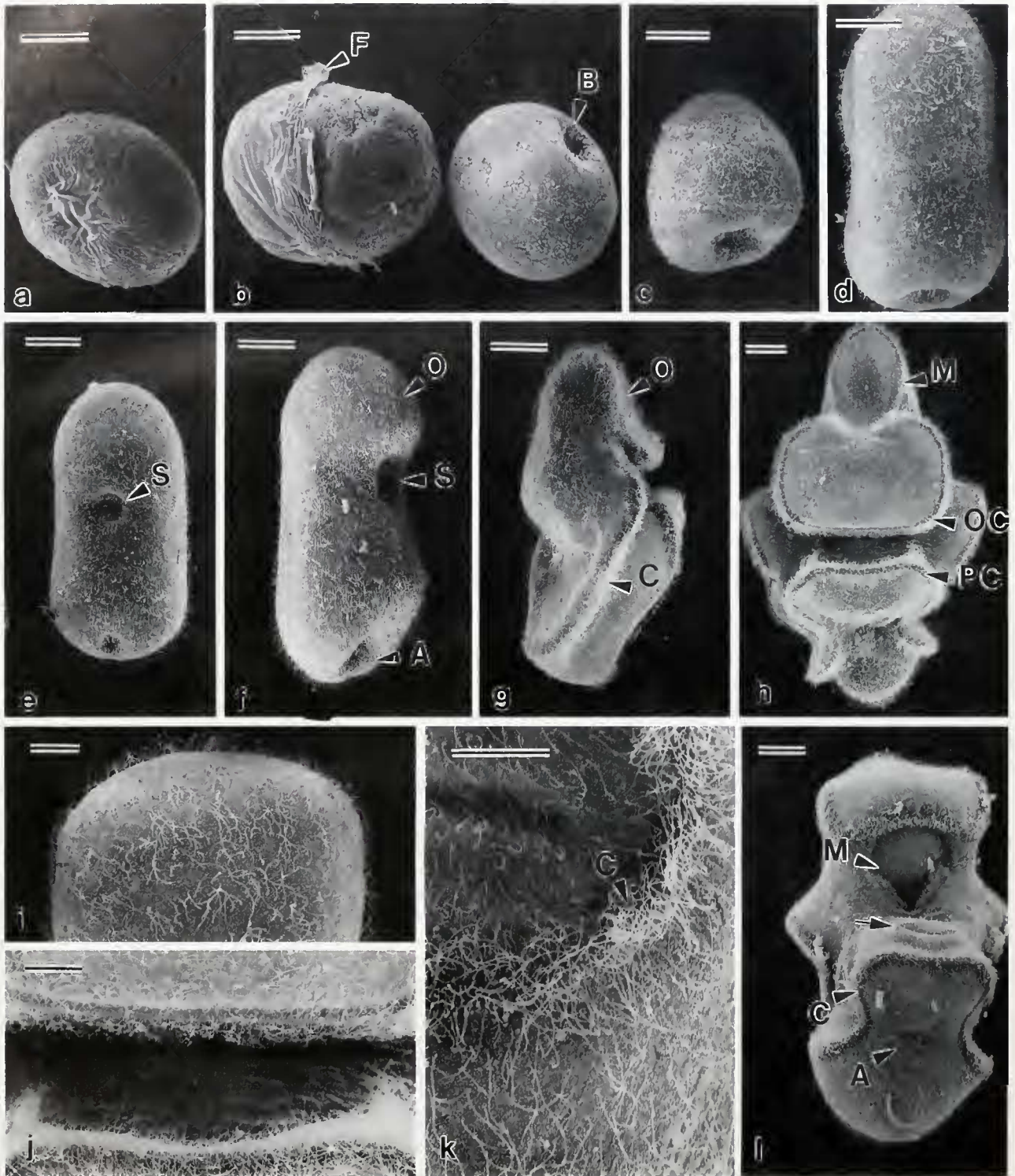


Figure 3. SEM of development through the bipinnaria stage. a. Late blastula within the fertilization membrane. b. Hatching and newly hatched gastrulae. B, blastopore; F, fertilization membrane. c. Gastrula starting to elongate. d. Elongate gastrula. e. Early bipinnaria forming the stomodeal invagination (S). f. Bipinnarial shape starting to develop, the stomodeum (S) has enlarged and the blastopore has moved to a ventral position to form the anus (A). O, forming oral hood. g. Bipinnaria side view with a distinct oral hood (O) and ciliary tracts (C). h. Late bipinnaria ventral view with oral- (OC) and postoral (PC) ciliary tracts. M, mouth. i. Ciliary field covering gastrula. j. Detail of bipinnaria in Figure 3h, showing the ciliary tracts around mouth and scattered cilia covering the larva. k. Ciliary tract (C) of a bipinnaria. l. Bipinnaria fixed in the dorsally-flexed position showing the plug-like structure on the post-oral surface (arrow). A, anus; M, mouth; C, ciliary tract. Scales: Fig. 3a-h, $l = 50 \mu\text{m}$; Fig. 3i-k = $20 \mu\text{m}$.

of the right enterocoel was not observed. By day 6, the ventral horn of the left posterior coelom forms and extends between the stomach and the intestine. Eight-day-old larvae are $790\ \mu\text{m}$ in length ($\pm 85\ \mu\text{m}$; $n = 20$).

Ten-day-old larvae are advanced bipinnaria (length, $990 \pm 150\ \mu\text{m}$; $n = 20$). By day 14, the ventral horn completes its growth fusing with the right enterocoel (Fig. 2f). In the preoral hood, the coelom extends anteriorly beyond the median-dorsal process, forming the lumen of the future median brachiolar arm. At the base of the median-dorsal process, the anterior coelom gives rise to two lateral extensions destined to be the coelomic lumina of the posterior brachiolar arms (Fig. 2e).

The larvae grow as advanced bipinnaria through the first month of development. By week five, early brachiolaria are present with three brachiolar arms or brachia. The longest brachium extends from the median-dorsal process and contains the main branch of the anterior coelom. On either side are two small brachia into which the lateral coelomic extensions grow. Each of these brachiolar arms are contractile. Advanced brachiolaria were present in eight-week-old cultures (Fig. 2h). These larvae have a well-developed brachiolar complex comprised of the three brachia and a centrally located adhesive disc. The adult primordium develops the posterior region of the brachiolaria (Fig. 2h, i). On the left side of the larvae, the five lobes of the hydrocoel are evident (Fig. 2h). The first adult spicules form as small rods positioned along each lobe of the hydrocoel (Fig. 2i). By week nine, the larvae were competent to metamorphose at a length of $1430\ \mu\text{m}$ ($\pm 194\ \mu\text{m}$; $n = 20$). This appears to be the upper growth limit of the larvae, as three-month-old brachiolaria were similar in length.

Metamorphosis

Advanced brachiolaria extend their arms and attach them to the bottom of the culture dishes in what appears to be searching behavior. The large median brachium bends at a 90° angle to the larval body, bringing the two posterior brachia and the adhesive disc into contact with the substratum. The larva then adhere temporarily to the bottom of the dish by means of the arm and then detach and continue swimming. To induce metamorphosis, glass slides with a primary algal film or natural shell substrata were placed in finger bowls, and competent larvae were introduced. The brachiolaria did not respond to the slides, but attached to the undersurfaces of the shells within a few hours of introduction. During temporary attachment, larvae moved over the surface of the substratum and exhibited searching behavior, with the brachiolar arms attaching and detaching as the larvae "walked" over the substratum. Following this exploratory phase, the larvae ceased to move, attached permanently with their brachia

and adhesive disc, and started to metamorphose. During metamorphosis, the larval body is shortened and resorbed to a thin stalk. The adult primordium develops with formation of a pentamerous shape. The hydrocoel expands, and the first adult tube feet form on the oral surface. These tube feet are used for attachment and locomotion. Eventually, the post-larvae break free of their attachment stalks taking up an independent existence at a diameter of $450\text{--}500\ \mu\text{m}$ five to six days after settlement (Fig. 2j). Development continues with completion of the adult digestive tract. Newly detached post-larvae do not have a mouth or an anus.

Scanning electron microscopy

Examination of the surface of hatching gastrulae of *Paritriella regularis* shows that they are covered by a uniform field of cilia (Fig. 3b). The wrinkled appearance of the fertilization membrane is probably due to the collapse of the membrane during fixation and drying (Fig. 3a, b). On hatching, the gastrulae start to elongate (Fig. 3b–d, i). With the development of the bipinnaria, pre- and postoral portions of the larvae are evident with a slight depression between them where the stomodeal invagination arises (Fig. 3e, f). As the larvae grow, the bipinnarial processes and the pre- and postoral ciliary tracts form (Fig. 3g, h). These tracts, a conspicuous feature of the larvae, are sinuous ridges of dense cilia that follow the contours of the bipinnarial processes (Fig. 3j, k). In addition to the ciliary tracts, the bipinnaria are also covered by a uniform field of cilia (Fig. 3j, k). Bipinnaria preserved in the dorsally flexed position reveal the presence of a plug-like structure on the postoral surface (Fig. 3l). On contraction of the larva, this structure would function as a seal over the mouth.

Formation of the brachiolar complex is evident with the appearance of the median brachiolar arm and two small lateral projections (Fig. 4a). Ridges on the median arm are developing papillae (Fig. 4a). In advanced brachiolaria, the arms take on their distinctive shape and are covered by adhesive papillae (Fig. 4b, c). An adhesive disc is positioned at the base of the arms (Fig. 5a). Like the bipinnaria, the brachiolaria has ciliary tracts and is covered by cilia (Fig. 4b, c). In addition to the preoral ciliary tract on the median-dorsal process, a lateral ciliary tract is present along the median brachium (Fig. 4b, i, j).

Preservation of the brachiolaria larvae differed with the two fixation methods used. An external coat covers the adhesive surface of the arms of brachiolaria fixed with the Bouin's method (Fig. 4a–j), whereas this coat is not present in larvae fixed with the glutaraldehyde-seawater method (Fig. 5a–h). The coat is a thin mesh-like material on the surface of the brachia that gives the arms a smooth appearance (Fig. 4b–j). Due to contraction of the larvae, it

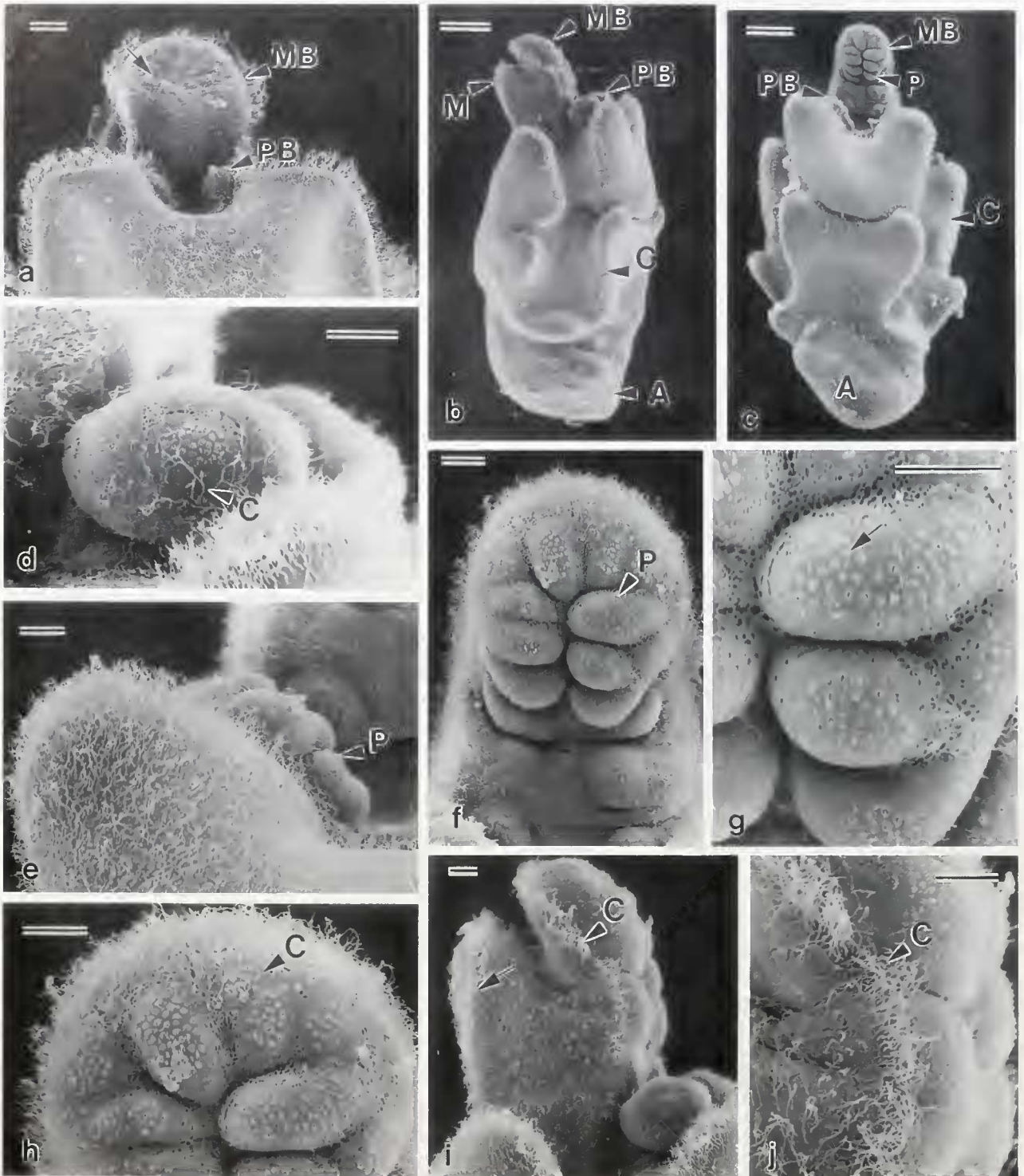


Figure 4. SEM of development through the brachiolaria stage of larvae fixed initially with Bouin's. a. Early brachiolaria. The two posterior arms (PB) are starting to form at the base of the median brachium (MB). Ridges (arrow) on the surface of the median brachium are developing papillae. b. Brachiolaria side view. The median brachium (MB) emerges from the median-dorsal process (M) and the posterior brachia (PB). A, developing adult primordium; C, ciliary tract. c. Late brachiolaria ventral view. Note the smooth surface of the median brachium (MB), which has two rows of papillae (P), and the cilia covering of the larva. The adult primordium (A) region is evident posteriorly. PB, posterior brachium. d. Detail of the brachiolaria shown in Figure 2b. The posterior brachium has a smooth extracellular coat through which the cilia (C) emerge. e. Detail of the advanced brachiolaria shown in Figure 2c showing the posterior brachium and

is not possible to determine whether the adhesive disc has an extracellular covering. The rest of the brachiolar surface does not have this coat (Fig. 4b–j).

The removal of the extracellular coat by the glutaraldehyde-seawater method reveals the underlying structure of the brachiolar complex (Fig. 5a–g). Papillae cover the brachia down to their bases and surround the adhesive disc (Fig. 5a, b). The median brachium is considerably longer than the other two and has 9–16 papillae arranged in two rows. A cluster of papillae covers the surface of the posterior arms (Fig. 5c). Nodular arrays of raised epithelial cells dot the surface of the papillae (Fig. 5b,d–f). In side-view, these nodules are raised structures that have a fuzzy tip, probably comprised of microvilli (Fig. 5e). In brachiolaria with an intact extracellular coat, small elevations of the coat indicate the position of the underlying nodules (Fig. 4g, h). Cilia on and around the papillae occasionally protrude through the glycocalyx (Figs. 4d, h; 5d, e). The adhesive disc is a round, flat structure with raised epithelial cells similar to those seen on the papillae (Fig. 5b, g). In larvae fixed with the glutaraldehyde-seawater method, smooth patches of material apparently secreted by the papillae are evident on the surface of the brachia (Fig. 5c).

Competent brachiolaria have a distinct adult rudiment at the posterior end of the larvae and scattered cilia cover the future aboral surface (Fig. 5h). The reduction of the larval body to a thin attachment stalk is shown in the wispy tissue attached to the metamorphosing larva in Figure 5i. Post-larvae have two pairs of tube feet per arm, and cilia are present on the epidermis (Fig. 5j).

Discussion

Development of *Patiriella regularis* is similar to other asteroids that develop indirectly through planktotrophic bipinnaria and brachiolaria larvae (Dan, 1968; Strathmann, 1987). The bipinnarial processes of *P. regularis* larvae, characteristic of spinulosan asteroids, are relatively short in comparison with those of forcipulate larvae, which develop into long and slender extensions of the larval body (Gemmill, 1914; Strathmann, 1971; Barker, 1978b).

The wrinkled blastula has been widely reported in asteroid embryology for both indirect and direct developers (Mortensen, 1921; Chia, 1968; Komatsu, 1972, 1976;

Oguro *et al.*, 1976; Byrne, 1991). A wrinkled blastula occurs in *Patiriella regularis*, which has small eggs, and it also occurs in the Australian species, *P. exigua*, *P. calcar*, and *P. gunnii*, which have large ova 350–400 μm in diameter (Lawson-Kerr and Anderson, 1978; Byrne, 1991). Blastular wrinkling in each of these *Patiriella* species results from the folding of the blastoderm into the blastocoel with subsequent smoothing out at the advanced blastula stage (Lawson-Kerr and Anderson, 1978; Byrne, 1991). In echinoids, wrinkled blastulae are only reported in species with large eggs (Williams and Anderson, 1975; Ame-miya and Tsuchiya, 1979; Raff, 1987; Parks *et al.*, 1989), and the wrinkled blastula may be a consequence of the shift from indirect to direct development (Raff, 1987; Parks *et al.*, 1989). There is no evidence of a relationship between blastular wrinkling and egg size in asteroids, and infolding of the blastoderm may be associated with the mechanics of cleavage (Anderson, pers. comm.). Up to the early blastula stage, cleavage gives rise to large cuboidal blastomeres held within a close-fitting fertilization membrane. As development continues, the embryo may not be able to accommodate additional cuboidal cells in a spherical shape due to insufficient space within the fertilization membrane, resulting in the onset of wrinkling. In asteroids and echinoids that have a wrinkled blastula in their development, smoothing of advanced blastula corresponds with the transition from a cuboidal to a columnar blastomere organization (Parks *et al.*, 1989; Byrne, pers. obs.). Compared with cuboidal blastomeres, this columnar organization may be more readily accommodated in a spherical shape. But not all asteroids have a wrinkled blastula (Dan, 1968; Strathmann, 1987), and for these species, the spatial relationship between the blastular surface and the fertilization membrane during the cuboidal-columnar transition should be documented. Recent work suggests that wrinkling of lecithotrophic echinoid embryos may also be a mechanical phenomenon (Henry, pers. comm.).

The posterior enterocoel that forms on the left side of the archenteron in the early bipinnaria of *Patiriella regularis* is not a general feature of asteroid embryology (Dan, 1968; Strathmann, 1987). Homologous structures are reported in the bipinnaria of *Asterias rubens* and *Marthasterias glacialis*, where similar masses of cells may arise on the right or left side of the archenteron (Gemmill,

papillae (P). The arm has an extracellular coat that gives it a smooth appearance. f. Median brachium and papillae (P). The arm has an extracellular coat that gives it a smooth appearance. g. Detail of the papillae of the median brachium and the smooth mesh-like extracellular coat. Raised bumps on the papillae (arrow) indicate the position of underlying raised epithelial cells (see Fig. 5b). h. Papillae at the tip of the median brachium with cilia (C) emerging through the surface coat. i. Anterior portion of brachiolaria shown in Figure 2b, showing the pre-oral ciliary tract on the median dorsal process (arrow) and the ciliary tract (C) on the median brachium. j. Detail of the ciliary tract (C) along the median brachium. Scales: Fig. 4a, d–j = 20 μm ; Fig. 4b, c = 100 μm .

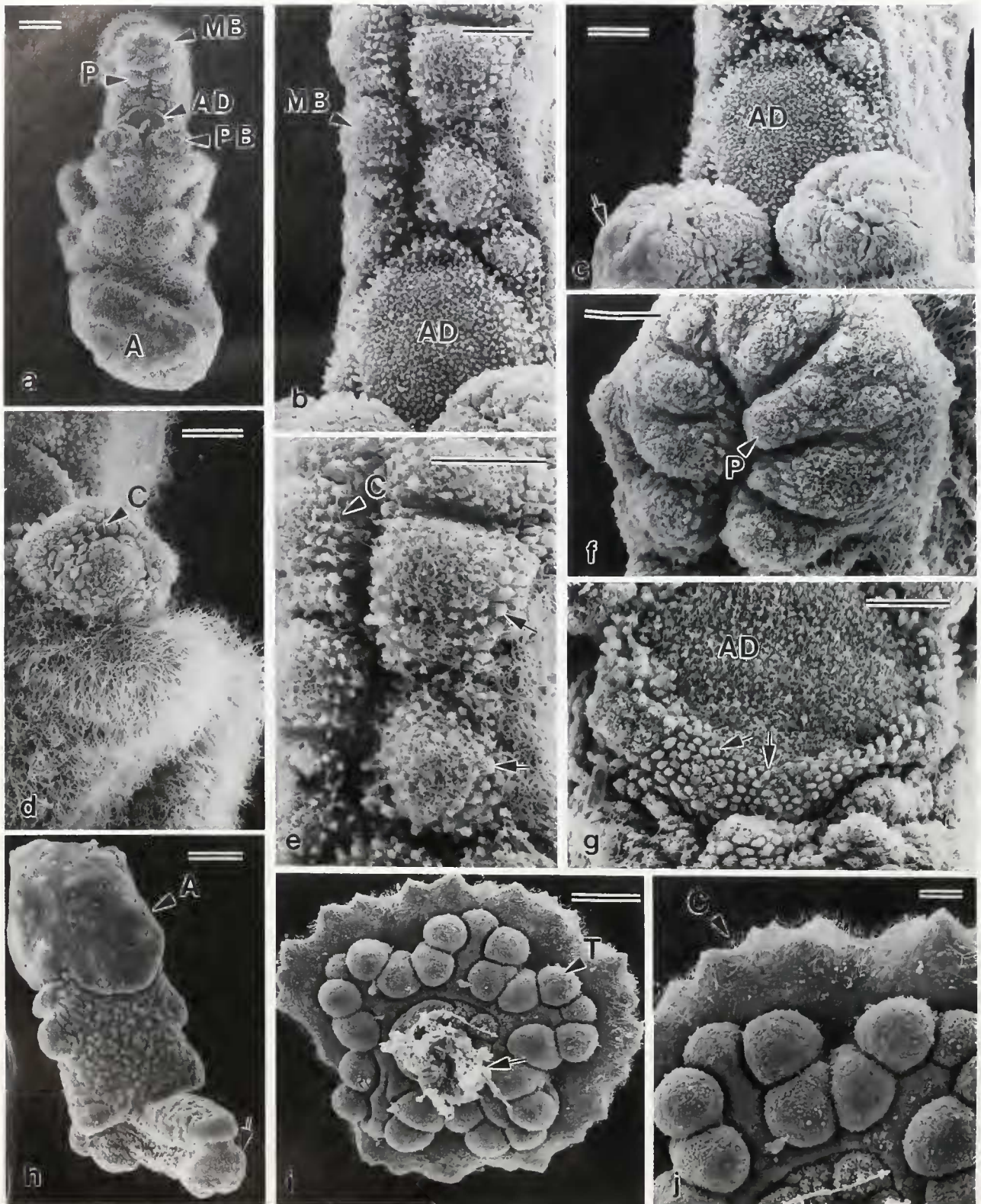


Figure 5. SEM of development through metamorphosis of larvae fixed by the glutaraldehyde-seawater method. a. Brachiolaria ventral view. Note the median (MB) and posterior brachia (PB) and the adhesive disc (AD). Cilia cover the larva, and the adult primordium is evident posteriorly (A). b. Median brachium (MB) with two rows of papillae. Note the absence of an extracellular coat. The papillae and adhesive disc (AD) are dotted by raised epithelial cells. c. Posterior brachium with a patch of secreted material on its surface (arrow). AD, Adhesive disc. d. Detail of the brachiolaria shown in Figure 5a. Cilia cover the larva and are also present on and around the papillae (C). Note the absence of an extracellular coat on the posterior

1914). In these species, this cell mass detaches from the gut and either breaks up into mesoderm or fuses with the advancing right or left enterocoel; in *M. glacialis* a central cavity occasionally forms (Gemmill, 1914). A similar situation to that seen in *P. regularis* occurs in the other asterinid species *Asterina miniata* and *A. pectinifera* (Heath, 1917; Newman, 1925; Komatsu, pers. comm.). As in *P. regularis*, a posterior enterocoelic growth arises on the left side of the archenteron of *A. miniata* and *A. pectinifera*, forming a third enterocoel that fuses with the anterior left enterocoel (Heath, 1917; Newman, 1925). In *P. regularis* and *A. miniata*, the posterior enterocoel is a functionally important structure that grows during development and gives rise to the posterior coelom (Newman, 1925). This contrasts with typical asteroid development, where the posterior coelom is derived from partition of the left anterior enterocoel (Gemmill, 1914; Dan, 1968; Strathmann, 1987). There is speculation as to the significance of the presence of a posterior enterocoelic growth and a third enterocoel (Gemmill, 1914; Heath, 1917; Newman, 1925). In the development of *A. miniata*, Newman (1925) considered the posterior enterocoel to be a vestigial feature. Gemmill (1914) and Heath (1917) considered the thickening of the archenteron wall in some asteroids, and the third enterocoel in others, to be rudiments of a posterior coelom present in the larvae of a common enteropneust-echinoderm ancestor. Thus, as suggested by Gemmill (1914), the posterior enterocoel of *P. regularis* may be homologous to the trunk coelom in enteropneust embryology.

The plug-like structure on the post-oral surface of the bipinnaria of *Patiriella regularis* has not been described before. This structure is evident only in bipinnaria fixed in the dorsally flexed posture and appears to serve as a seal for the mouth on ventral contraction of the larvae. In this manner it may function as a mechanism to prevent undesirable particles from entering the mouth. It was not seen in live specimens examined with the light microscope. Although this mouth seal has not been reported in the bipinnaria of other asteroids, its presence may be revealed by scanning electron microscopy.

The ultrastructure of the brachiolar complex has been described for the forcipulate asteroids *Stichaster australis* and *Coscinasterias calamaria* (Barker, 1978a). In comparison with these species, the median brachiolar arm of *Patiriella regularis* is well-supplied with adhesive papillae.

In *S. australis* and *C. calamaria*, adhesive papillae are limited to the tip of the brachia, and the stem of the median brachium is smooth (Barker, 1978a). Like the brachiolaria of these species, cilia are also present on the papillae of *P. regularis* and may have a sensory role in the location of suitable substrata for settlement (Barker, 1978a). The raised epithelial nodules on the brachial papillae and adhesive disc of *P. regularis* appear to correspond to the batteries of secretory cells revealed by transmission electron microscopy of the brachiolar complex of *S. australis* and *C. calamaria* (Barker, 1978a).

Temporary attachment of *P. regularis* brachiolaria is achieved by the median brachium as it extends over substratum, assisted by adhesion of the posterior arms. The patches of smooth material on the brachia may be used for adhesion. For *P. regularis*, as reported for *S. australis* and *C. calamaria* (Barker, 1978a), it appears that permanent attachment is achieved by secretion of a cement-like material by the adhesive disc. Early descriptions of brachiolaria refer to the attachment disc as a 'sucker' because it was thought to effect attachment by means of suction with the edge of the disc forming a seal (Gemmill, 1914; Mortensen, 1921).

The difference in preservation of the brachiolaria by the two fixation methods is striking. Brachiolaria fixed initially with Bouin's fluid have a glycocalyx-like material covering their brachia, whereas larvae fixed with the glutaraldehyde-seawater method do not. Removal of surface coats by conventional fixation methods is reported for several echinoderms (Cameron and Holland, 1983; McKenzie, 1987). An extracellular coat similar to that on the brachiolar complex of *P. regularis* is present on the larvae of *Asterina miniata* (Cameron and Holland, 1983). In *A. miniata*, however, this coat covers the entire surface of the larva (Cameron and Holland, 1983). The preservation of a glycocalyx on the brachia, but not on the rest of the larval surface of *P. regularis*, suggests that it may function in association with the brachiolar complex as a protective covering for the attachment surface.

The searching behavior, settlement, and metamorphosis of *Patiriella regularis* is characteristic of asteroid brachiolaria (Gemmill, 1914; Barker, 1977). In contrast to that reported for *Coscinasterias calamaria*, the presence of a primary algal film is not sufficient to induce metamorphosis of *P. regularis* (Barker, 1977). The attachment and metamorphosis of the brachiolaria on the undersides of

brachium. e. Detail of the median brachium showing raised epithelial cells (arrows) on the papillar surface. Cilia are present on and around the papillae (C). f. Papillae (P) at the tip of the median brachium. g. Adhesive disc (AD), the arrows point to raised epithelial cells. h. Late brachiolaria ventral view. The larva is in the exploratory/attachment posture with the median brachium extended 90° to the larval body (arrow). A, adult primordium. i. Metamorphosing larva detached from its stalk, which appears as wispy material (arrow). Two pairs of tube feet (T) are present in each radius. j. Detail of the metamorphosing larva. The future adult surface is covered by cilia (C). Scales: Fig. 5a, h, i = 50 μ m; Fig. 5b–g, j = 20 μ m.

shells and not on the film-covered slides, suggests that shade and a rough-textured surface may be an important factor in selecting a site for metamorphosis in this species. Crump (1969) also reported attachment and metamorphosis of *P. regularis* brachiolaria on the undersides of introduced substrata.

The observation of simultaneous spawning of male and female *Patiriella regularis* in the field is similar to that reported for several asteroids (Minchin, 1987; Pearse *et al.*, 1988). The nearest distance between spawning *P. regularis*, however, is considerably longer than for *Marthasterias glacialis*, which gathers in spawning assemblages prior to gamete release (Minchin, 1987). Although some of the *P. regularis* releasing gametes were 0.5 m from an adjacent spawner, several individuals appeared to be spawning in isolation, as reported by Pearse *et al.* (1988). At this distance, and particularly for those individuals spawning in isolation, gamete dilution would reduce the chance of fertilization. Echinoid zygote production in the field decreases dramatically if females are more than 20 cm apart from spawning males (Pennington, 1985). There is evidence, however, that asteroid sperm and sperm of other echinoderms are attracted to conspecific ova, and this, to some extent, may ameliorate the problem of gamete dilution (Miller, 1989; Byrne, 1990). The collection site is subject to strong currents, and it seems that the chances of fertilization would be enhanced by the slack water conditions that coincided with spawning, as noted for breeding in holothuroids (McEuen, 1988).

From laboratory culture at 18–22°C, the pelagic period of *Patiriella regularis* has a duration of 9–10 weeks; it may be longer in the field, where ambient sea surface temperatures of 16–18°C in Otago Harbour coincide with the planktonic period of this species. This duration of larval life is similar to that of other temperature planktotrophic asteroids, although it is somewhat shorter than that of asteroids from the northern Pacific, where ambient temperatures range from 7 to 13°C (Strathmann, 1978b). Latitudinal differences in larval life undoubtedly reflect differences in ambient temperature, with the longest planktonic period of 22 weeks recorded for the antarctic asteroid *Odontaster validus* at sea temperatures of –2––1°C (Pearse and Bosch, 1986).

Development of *Patiriella regularis* through feeding bipinnaria and brachiolaria larvae is typical of asteroid embryogenesis and contrasts with the development of the Australian *Patiriella*. *P. exigua*, *P. pseudoexigua*, *P. calcar*, and *P. gunnii* have completely lost the bipinnarial stage and develop directly through a non-feeding brachiolaria (Mortensen, 1921; Lawson-Kerr and Anderson, 1978; Byrne, 1991; Chen and Chen, 1991). Also in contrast to *P. regularis*, these species have large ova, the evolution of which is considered to be a pre-adaptive trait for the shift to direct development (Chia, 1968). Larvae

derived from such eggs would no longer be obligate planktotrophs, resulting in the loss of structures required for feeding (Strathmann, 1978a). The development of *P. regularis* provides the basic reference for comparison with the direct developers. Features that are particularly important for comparison include the mode and timing of archenteron and coelom formation in *P. regularis*, and the morphology of larval feeding structures. As documented for echinoids (Raff, 1987), the evolution of direct development in *Patiriella* may involve heterochronic changes in these features. Together with the developmental chronologies of the other *Patiriella* species, the ontogeny of *P. regularis* presented here will be used to determine the changes underlying the shift to direct development within the genus and to assess the pathways by which feeding larvae were lost. The use of *Patiriella* as a tool with which to examine developmental processes in evolution is the subject of ongoing research.

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